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(54) Title: **HYBRID BIOLOGIC/SYNTHETIC POROUS EXTRACELLULAR MATRIX SCAFFOLDS**



(57) Abstract: Methods of making a hybrid biologic/synthetic scaffold for repairing damaged or diseased tissue are provided. The methods include the step of suspending pieces of an extracellular matrix material in a liquid to form a slurry, and coating a synthetic mat with the slurry, or mixing or layering the slurry with a synthetic polymer solution. The liquid is subsequently driven off so as to form a foam. Porous implantable scaffolds fabricated by such a method are also disclosed.

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HYBRID BIOLOGIC/SYNTHETIC POROUS EXTRACELLULAR MATRIX SCAFFOLDS

CROSS REFERENCE TO RELATED APPLICATIONS

5 Cross reference is made to copending U.S. patent applications Serial No. XX/XXX,XXX entitled "Meniscus Regeneration Device and Method" (Attorney Docket No. 265280-71141, DEP-745); Serial No. XX/XXX,XXX entitled "Devices from Naturally Occurring Biologically Derived Materials" (Attorney Docket No. 265280-71142, DEP-748); Serial No. XX/XXX,XXX entitled "Cartilage Repair
10 Apparatus and Method" (Attorney Docket No. 265280-71143, DEP-749); Serial No. XX/XXX,XXX entitled "Unitary Surgical Device and Method" (Attorney Docket No. DEP-750); Serial No. XX/XXX,XXX entitled "Cartilage Repair and Regeneration Device and Method" (Attorney Docket No. 265280-71145, DEP-752); Serial No. XX/XXX,XXX entitled "Porous Extracellular Matrix Scaffold and Method"
15 (Attorney Docket No. 265280-71146, DEP-747); Serial No. XX/XXX,XXX entitled "Cartilage Repair and Regeneration Scaffolds and Method" (Attorney Docket No. 265280-71180, DEP-763); and Serial No. XX/XXX,XXX entitled "Porous Delivery Scaffold and Method" (Attorney Docket No. 265280-71207, DEP-762), each of which is assigned to the same assignee as the present application, each of which is
20 filed concurrently herewith, and each of which is hereby incorporated by reference. Cross reference is also made to U.S. Patent Application Serial No. 10/172,347 entitled "Hybrid Biologic-Synthetic Bioabsorbable Scaffolds" which was filed on June 14, 2002, which is assigned to the same assignee as the present application, and which is hereby incorporated by reference.

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TECHNICAL FIELD OF THE DISCLOSURE

The present disclosure relates generally to an extracellular matrix, scaffold, and more particularly to a porous extracellular matrix scaffold for repairing or regenerating body tissue and a method for making such a scaffold.

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BACKGROUND AND SUMMARY OF THE INVENTION

Naturally occurring extracellular matrices (ECMs) are used for tissue repair and regeneration. One such ECM is small intestine submucosa (SIS). SIS has

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been used to repair, support, and stabilize a wide variety of anatomical defects and traumatic injuries. Commercially available SIS material is derived from porcine small intestinal submucosa that remodels the qualities of its host when implanted in human soft tissues. Further, it is taught that the SIS material provides a natural matrix with a three-dimensional microstructure and biochemical composition that facilitates host cell proliferation and supports tissue remodeling. SIS products, such as Oasis material and Surgisis material, are commercially available from Cook Biotech, Bloomington, IN.

An SIS product referred to as RESTORE Orthobiologic Implant is available from DePuy Orthopaedics, Inc. in Warsaw, Indiana. The DePuy product is described for use during rotator cuff surgery, and is provided as a resorbable framework that allows the rotator cuff tendon to regenerate itself. The RESTORE Implant is derived from porcine small intestine submucosa that has been cleaned, disinfected, and sterilized. Small intestine submucosa (SIS) has been described as a naturally occurring ECM composed primarily of collagenous proteins. Other biological molecules, such as growth factors, glycosaminoglycans, etc., have also been identified in SIS. See Hodde et al., *Tissue Eng.* 2(3): 209-217 (1996); Voytik-Harbin et al., *J. Cell Biochem.*, 67:478-491 (1997); McPherson and Badylak, *Tissue Eng.*, 4(1): 75-83 (1998); Hodde et al., *Endothelium*, 8(1):11-24 (2001); Hodde and Hiles, *Wounds*, 13(5): 195-201 (2001); Hurst and Bonner, *J. Biomater. Sci. Polym. Ed.*, 12(11) 1267-1279 (2001); Hodde et al., *Biomaterial*, 23(8): 1841-1848 (2002); and Hodde, *Tissue Eng.*, 8(2): 295-308 (2002), all of which are incorporated by reference herein. During seven years of preclinical testing in animals, there were no incidences of infection transmission from the implant to the host, and the RESTORE Implant has not decreased the systemic activity of the immune system. See Allman et al., *Transplant*, 17(11): 1631-1640 (2001); Allman et al., *Tissue Eng.*, 8(1): 53-62 (2002).

While small intestine submucosa is available, other sources of submucosa are known to be effective for tissue remodeling. These sources include, but are not limited to, stomach, bladder, alimentary, respiratory, or genital submucosa, or liver basement membrane. See, e.g., U.S. Patents Nos. 6,379,710, 6,171,344, 6,099,567, and 5,554,389, hereby incorporated by reference. Further, while SIS is most often porcine derived, it is known that these various submucosa materials may

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be derived from non-porcine sources, including bovine and ovine sources.

Additionally, the ECM material may also include partial layers of laminar muscularis mucosa, muscularis mucosa, lamina propria, stratum compactum and/or other tissue materials depending upon factors such as the source from which the ECM material was derived and the delamination procedure.

For the purposes of this invention, it is within the definition of a naturally occurring ECM to clean, delaminate, and/or comminute the ECM, or even to cross-link the collagen fibers within the ECM. It is also within the definition of naturally occurring ECM to fully or partially remove one or more sub-components of the naturally occurring ECM. However, it is not within the definition of a naturally occurring ECM to separate and purify the natural collagen or other components or sub-components of the ECM and reform a matrix material from the purified natural collagen or other components or sub-components of the ECM. While reference is made to SIS, it is understood that other naturally occurring ECMs (e.g., stomach, bladder, alimentary, respiratory, and genital submucosa, and liver basement membrane), whatever the source (e.g., bovine, porcine, ovine) are within the scope of this disclosure. Thus, in this application, the terms "naturally occurring extracellular matrix" or "naturally occurring ECM" are intended to refer to extracellular matrix material that has been cleaned, disinfected, sterilized, and optionally cross-linked. The terms "naturally occurring extracellular matrix" and "naturally occurring ECM" are also intended to include ECM foam material prepared as described in copending U.S. Patent Application Serial No. XX/XXX,XXX entitled "Porous Extracellular Matrix Scaffold and Method" (Attorney Docket No. 265280-71146, DEP-747), filed concurrently herewith.

The following U.S. patents, hereby incorporated by reference, disclose the use of ECMs for the regeneration and repair of various tissues: 6,187,039; 6,176,880; 6,126,686; 6,099,567; 6,096,347; 5,997,575; 5,968,096; 5,955,110; 5,922,028; 5,885,619; 5,788,625; 5,762,966; 5,755,791; 5,753,267; 5,711,969; 5,645,860; 5,641,518; 5,554,389; 5,516,533; 5,445,833; 5,372,821; 5,352,463; 5,281,422; and 5,275,826.

The manipulation of scaffold pore size, porosity, and interconnectivity is of emerging importance in the field of tissue engineering (Ma and Zhang, 2001, J. Biomed Mater Res. 56(4): 469-477; Ma and Choi, 2001, Tissue Eng., 7(1):23-33),

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because it is believed that the consideration of scaffold pore size and density/porosity influences the behavior of cells and the quality of tissue regenerated. In fact, several researchers have shown that different pore sizes influence the behavior of cells in porous three-dimensional matrices. For example, it has been demonstrated in the art that for adequate bone regeneration to occur scaffold pore size should to be at least 100 microns (Klawitter et al., 1976, J Biomed Mater Res, 10(2):311-323). For pore sizes and interconnectivity less than that, poor quality bone is regenerated, and if pore size is between 10-40 microns bone cells are able to form only soft fibro-vascular tissue (White and Shors, 1991, Dent Clin North Am, 30:49-67). The current consensus of research for bone regeneration indicates that the requisite pore size for bone regeneration is 100-600 microns (Shors, 1999, Orthop Clin North Am, 30(4):599-613; Wang, 1990, Nippon Seikeigeka Gakki Zasshi, 64(9):847-859). It is generally accepted that optimal bone regeneration occurs for pore sizes between 300-600 microns.

Similarly, for the regeneration of soft orthopaedic tissues, such as ligament, tendon, cartilage, and fibro-cartilage, scaffold pore size is believed to have a substantial effect. For example, basic research has shown that cartilage cells (chondrocytes) exhibit appropriate protein expression (type II collagen) in scaffolds with pore sizes of the order of 20 microns and tend to dedifferentiate to produce type I collagen in scaffolds with nominal porosity of about 80 microns (Nehrer et al., 1997, Biomaterials, 18(11):769-776). More recently, it has been shown that cells that form ligaments, tendons, and blood vessels (fibroblasts and endothelial cells) exhibit significantly different activity when cultured on scaffolds with differing pore sizes ranging from 5 to 90 microns (Salem et al., 2002, J Biomed Mater Res, 61(2):212-217).

Copending U.S. Application Serial No. XX/XXX,XXX entitled "Porous Extracellular Matrix Scaffold and Method" (Attorney Docket No. 265280-71146, DEP-747), filed contemporaneously herewith and hereby incorporated by reference, describes methods for making ECM foams wherein the porosity is controlled. Using the methods so described, ECM foams are made having the desired porosity for a particular application.

In some applications, it is also desirable to control the rate of resorption of the scaffold. It is known in the art to make implantable three-

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dimensional synthetic scaffolds with controlled porosity and controlled resorption rates. See, e.g., U.S. Patents Nos. 6,333,029 and 6,355,699, hereby incorporated by reference. These synthetic foams may be isotropic in form, or may be anisotropic, providing various gradient architectures.

5 In addition to synthetic foams, it is known that resorption rates of an implant may be controlled by providing a synthetic portion comprising a perforated or non-perforated sheet or a mat with a woven, knitted, warped knitted (i.e., lace-like), nonwoven, or braided structure. It is understood that in any of the above structures, mechanical properties of the material can be altered by changing the density or texture
10 of the material. The fibers used to make the reinforcing component can be for example, monofilaments, yarns, threads, braids, or bundles of fibers. These fibers can be made of any biocompatible material. In an exemplary embodiment, the fibers that comprise the nonwoven or three-dimensional mesh are formed of a polylactic acid (PLA) and polyglycolic acid (PGA) copolymer at a 95:5 mole ratio. Illustrated
15 examples of the synthetic portion also include 90/10 PGA/PLA, 95/5 PLA/PGA, and polydioxanone (PDO) nonwoven mats, and perforated thin sheets of 60/40 PLA/PCL (polycaprolactone) or 65/35 PGA/PCL.

A variety of biocompatible polymers can be used to make fibers for the synthetic portion. Examples of suitable biocompatible, bioabsorbable polymers that
20 could be used include polymers selected from the group consisting of aliphatic polyesters, poly(amino acids), copoly(ether-esters), polyalkylenes oxalates, polyamides, poly(iminocarbonates), polyorthoesters, polyoxaesters, polyamidoesters, polyoxaesters containing amine groups, poly(anhydrides), polyphosphazenes, biomolecules and blends thereof. For the purpose of this disclosure aliphatic
25 polyesters include but are not limited to homopolymers and copolymers of lactide (which includes lactic acid, D-, L- and meso lactide), glycolide (including glycolic acid), ϵ -caprolactone, p-dioxanone (1,4-dioxan-2-one), trimethylene carbonate (1,3-dioxan-2-one), alkyl derivatives of trimethylene carbonate, δ -valerolactone, β -butyrolactone, γ -butyrolactone, ϵ -decalactone, hydroxybutyrate (repeating units),
30 hydroxyvalerate (repeating units), 1,4-dioxepan-2-one (including its dimer 1,5,8,12-tetraoxacyclotetradecane-7,14-dione), 1,5-dioxepan-2-one, 6,6-dimethyl-1,4-dioxan-2-one 2,5-diketomorpholine, pivalolactone, α,α -diethylpropiolactone, ethylene

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carbonate, ethylene oxalate, 3-methyl-1,4-dioxane-2,5-dione, 3,3-diethyl-1,4-dioxan-2,5-dione, 6,8-dioxabicyclooctane-7-one, copolymers, and polymer blends thereof.

Other synthetic polymers are known in the art and may be used within the scope of this disclosure.

5 The particular polymer may be selected depending on one or more of the following factors: (a) bio-absorption (or bio-degradation) kinetics; (b) in-vivo mechanical performance; and (c) cell response to the material in terms of cell attachment, proliferation, migration and differentiation and (d) biocompatibility.

With respect to the bio-absorption kinetics, it is known to control resorption rates by
10 selection of the polymer or copolymer. By way of example, it is known that a 35:65 blend of ϵ -caprolactone and glycolide is a relatively fast absorbing polymer and a 40:60 blend of ϵ -caprolactone and (L)lactide is a relatively slow absorbing polymer. Optionally, two or more polymers or copolymers could then be blended together to form a foam having several different physical properties.

15 In some orthopaedic applications, it is desirable to combine the tissue remodeling properties of ECM with the controlled resorption properties of synthetic foams, mats, or sheets. Thus, methods are provided for making porous scaffolds for the repair or regeneration of a body tissue, wherein the scaffolds comprise an ECM component and a synthetic portion. According to one illustrative embodiment, there
20 is provided a method of making an implantable scaffold for repairing damaged or diseased tissue. The method includes the steps of suspending pieces of an ECM material in a liquid and mixing a polymer solution into the liquid. The mixture is formed into a mass and, subsequently, the liquid is driven off so as to form interstices in the mass. In another embodiment, the method includes suspending pieces of an
25 ECM material in a liquid and forming a mass. A polymer mat, for example, a mesh or nonwoven, is coated with the ECM material, and, subsequently, the liquid is driven off, forming a foam having a combination of mechanical and biological features.

In one specific implementation of an exemplary embodiment, the liquid is driven off by lyophilizing the ECM and synthetic material and the liquid in
30 which they are suspended. In such a manner, the liquid is sublimed thereby forming the interstices in the mass.

The material density and pore size of the scaffold may be varied by controlling the rate of freezing of the suspension. The amount of water into which the

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pieces of extracellular matrix material are suspended may also be varied to control the material density and pore size of the resultant scaffold. Furthermore, as discussed above, the resorption rate may be controlled by varying the synthetic polymer structure or composition.

5 In accordance with another exemplary embodiment, there is provided an implantable scaffold for repairing or regenerating tissue prepared by the process described above.

Thus, one aspect of this disclosure is directed to a method of making an implantable scaffold for repairing or regenerating body tissue, the method
10 comprising the steps of suspending ECM material in a liquid to form a slurry, adding a synthetic portion to the slurry to make an ECM/synthetic composition, freezing the composition to form crystals therein, and driving off the crystals to form a foam. In one illustrated embodiment, the ECM is comminuted. In another illustrated embodiment, the liquid is water, the crystals are ice, and the crystals are driven off by
15 lyophilization.

In another aspect of this disclosure an implantable scaffold for repairing or regenerating body tissue is provided, the scaffold comprising a porous ECM foam and a synthetic mat imbedded therein.

Yet another aspect is an implantable scaffold comprising a mass of
20 ECM intermixed with a fibrous synthetic portion in a composition dried to have a desired porosity.

Still, another aspect of this invention is an implantable scaffold comprising a porous foam comprising ECM and a synthetic portion distributed within the foam.

25 The above and other features of the present disclosure will become apparent from the following description and the attached drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

The detailed description particularly refers to the accompanying
30 figures in which:

Fig. 1 is a scanning electron micrograph showing the surface of a porous three-dimensional SIS/synthetic polymer hybrid scaffold comprising a nonwoven, needled Vicryl™ sheet (Ethicon, Inc, Somerville, NJ) coated with SIS

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foam. Wet SIS slurry was coated on the Vicryl™ sheet, and the assembly was needled and then lyophilized;

Fig. 2 is a scanning electron micrograph showing the surface of a porous three-dimensional SIS/synthetic polymer hybrid scaffold in which the SIS
5 portion is sandwiched between two nonwoven Vicryl™ sheet. The assembly was needled and then lyophilized.

Fig. 3 is a scanning electron micrograph showing the surface of a porous three-dimensional SIS/synthetic polymer hybrid scaffold comprising a nonwoven, non-needled Vicryl™ sheet coated with SIS foam. Wet SIS slurry was
10 coated on the Vicryl™ sheet. The construct was centrifuged, additional SIS slurry was added, followed by additional centrifugation. The construct was needled and then lyophilized.

DETAILED DESCRIPTION OF THE DISCLOSURE

15 The present disclosure relates to porous scaffolds for implanting into the body of a patient to repair or regenerate damaged or diseased tissue. The porous scaffold is constructed from a naturally occurring extracellular material and a synthetic polymer. For example, the scaffold may be constructed from a mat of mesh or nonwoven synthetic material coated with SIS, a mixture of SIS and a synthetic
20 polymer, or from layers of SIS and synthetic polymer. The material density, pore size, and resorption rate of the porous scaffold may be varied to fit the needs of a given scaffold design.

Such porous scaffolds may be fabricated by first suspending pieces of an ECM material in a liquid. Suspension of the pieces of ECM material in the liquid
25 forms a mass in the form of, for example, a "slurry". The slurry may be used to coat a mat of mesh or nonwoven synthetic portion or a solution of a synthetic polymer may be added to the slurry, and the liquid may then be subsequently driven off of so as to form interstices therein. The liquid may be driven off in a number of different manners. For example, as will herein be described in greater detail, the liquid may be
30 driven off via sublimation in a freeze drying process. Alternatively, the liquid may also be driven off by subjecting the suspension to vacuum under a controlled heating process. The liquid may also be driven off from the suspension ultrasonically. Microwave energy may also be utilized to drive the liquid off of the suspension.

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Moreover, the liquid may include a water-soluble filler that is driven off, for example, by use of an alcohol.

While any of the aforementioned processes for driving off the liquid from the suspension may be used, along with any other process known by one skilled in the art, the processes of the present disclosure will herein be exemplary described in regard to a lyophilization process (i.e., freeze drying). However, it should be understood that such a description is merely exemplary in nature and that any one or more of the aforescribed processes for driving off the liquid from the suspension may be utilized to fit the needs of a given scaffold design or process design.

As discussed above, one useful process for fabricating the porous scaffolds of the present disclosure is by lyophilization. In this case, an ECM/polymer composition is frozen and subsequently lyophilized. Freezing the suspension causes the liquid to crystallize. These crystals are then sublimed during the lyophilization process, thereby leaving interstices in the material in the spaces previously occupied by the crystals. The material density and pore size of the resultant scaffold may be varied by controlling, among other things, the rate of freezing of the suspension and/or the amount of water in which the ECM material is suspended in at the start of the freezing process.

As a specific example of this process, fabrication of porous SIS/synthetic hybrid scaffolds by lyophilization will be described in detail. However, it should be appreciated that although the example is herein described in regard to an SIS/synthetic scaffold, fabrication of a scaffold constructed from other ECM materials and synthetic polymers may also be performed in a similar manner.

The first step in fabricating a porous scaffold with a desired pore size and density is the procurement of comminuted SIS. Illustratively, scissor-cut SIS runners (~6" long) are positioned in a 1700 series Comitrol™ machine, commercially available from Urschel Laboratories (Valpraiso, Indiana). The SIS material is processed and thereafter collected in a receptacle at the output of the machine. The material is then processed through the machine a second time under similar conditions. The resultant material is a "slurry" of SIS material (thin, long SIS fibers ~200 microns thick x 1-5 mm long) suspended in a substantially uniform manner in water. Although the suspension is herein described as being formed as a byproduct of the comminuting process, it should be appreciated that the pieces of SIS may be

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suspended in the liquid (i.e., water) in other manners known to those skilled in the art. Furthermore, while other methods are known for comminuting SIS, it is understood that for the purposes of the present disclosure, comminuted SIS comprises ribbon-like or string-like fibers wherein at least some of the individual pieces of ECM and SIS material have lengths greater than their widths and thicknesses. Such fibers may be interlaced to provide a felt-like material, if desired.

Process parameters can be varied using the above-identified 1700 series Comitrol™ machine, including the choice of blade used, whether water is used, the amount of water used, the speed at which the blades turn, and the number of times the material is passed through the machine. As an example, cutting head 140084-10 and a Vericut, sealed impeller from Urschel Laboratories may be used, with a flow of water of about two (2) gallons per minute, with the blade running at a constant speed of about 9300 rpm. A first pass through the machine at these parameters will produce fibrous SIS material of varying sizes, and a second pass will produce SIS fibers of a more uniform size. By way of example, the comminuted material may be tested to determine if it has the consistency of that which is desired for use in regard to the illustrative embodiments described herein by the following process: the comminuted SIS suspension or slurry is centrifuged, excess water is poured off and the remaining slurry is poured into a dish. By hand, a small amount of the comminuted SIS material in the dish is pinched between the thumb and index finger and gently lifted from the dish. Illustratively, at least a small amount of additional SIS, beyond the portion pinched between the thumb and index finger, will lift along with the material that has been pinched. This additional comminuted SIS material lifts with the material that is between the thumb and index finger because the individual pieces of comminuted SIS material are commingled or intertwined. Prior art methods of "comminuting" SIS using a freezer mill produce particles, rather than ribbon-like fibers. The prior art particles are not capable of significant intertwining and, for purposes of the present disclosure, are not included within the definition of comminuted SIS. See copending U.S. Patent Application Serial No. XX/XXX,XXX entitled "Porous Extracellular Matrix Scaffold and Method" (Attorney Docket No. 265280-71146, DEP-747).

A polymer solution is also prepared, as is known in the art. By way of example, a 95:5 weight ratio solution of 60/40 PLA/PCL is made and poured into a flask. The flask is placed in a water bath, stirring at 60-70°C for 5 hrs. The solution

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is filtered using an extraction thimble, extra coarse porosity, type ASTM 170-220 (EC) and stored in flasks.

Thereafter, the comminuted SIS suspension is mixed or layered with the polymer solution. In another embodiment, the SIS suspension is used with or without an intermixed polymer solution to coat a mat of mesh or nonwoven polymer. The SIS/polymer composition is frozen and lyophilized (i.e., freeze dried). In particular, the SIS/ polymer composition is frozen at a controlled rate of temperature drop to control the size of the formed crystals. Once frozen, and without allowing the material to thaw, the lyophilization process sublimates the crystals directly to a vapor under vacuum and low temperatures. This leaves voids or interstices in the spaces previously occupied by the crystals. In the embodiments wherein the polymer component is a mat of mesh or nonwoven material, the SIS forms a foam around the polymer component, and, depending on the size of the interstices, the foam may form therethrough.

Any method for freezing the composition to a desired temperature may be used. Likewise, any commercially available lyophilizer may be used for the lyophilization process. One exemplary machine for performing the lyophilization process is a Virtis Genesis™ Series lyophilizer that is commercially available from SP Industries, Inc. (Gardiner, New York).

The process parameters of the aforescribed fabrication process may be varied to produce scaffolds of varying pore sizes and material densities. For example, the rate at which the suspension is frozen, the amount of water present in the suspension, and the compactness of the ECM material each may be varied to produce scaffolds of varying pore sizes and material densities.

For instance, to produce scaffolds having a relatively large pore size and a relatively low material density, the composition may be frozen at a slow, controlled rate (e.g., $-1^{\circ}\text{C}/\text{min}$ or less) to a temperature of about -20°C , followed by lyophilization of the resultant mass. To produce scaffolds having a relatively small pore size and a relatively high material density, the SIS suspension may be tightly compacted by centrifuging the material to remove a portion of the liquid (e.g., water) in a substantially uniform manner prior to mixing with the polymer component. If desired, the fibers of the hybrid foams may be crosslinked, for example physically, chemically, or enzymatically, to increase mechanical strength of the scaffold.

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Additionally, because of the porosity of the scaffolds, the scaffolds of the present disclosure may be used to deliver various biologically active agents to a damaged tissue, in addition to those already present in the ECM, including one or more exogenous biologically derived agents or substances, one or more cell types, one or more biological lubricants, one or more biocompatible inorganic materials, one or more biocompatible synthetic polymers and one or more biopolymers. Various biologically active agents can be added to the foams, for example, prior to lyophilization, or subsequent to lyophilization by adsorption onto the surface or back filling into the foams after the foams are made. For example, the pores of the foam may be partially or completely filled with biocompatible resorbable synthetic polymers or biopolymers (such as collagen or elastin) or biocompatible inorganic materials (such as hydroxyapatite) and combinations thereof.

"Bioactive agents" include one or more of the following: chemotactic agents; therapeutic agents (e.g., antibiotics, steroidal and non-steroidal analgesics and anti-inflammatories, anti-rejection agents such as immunosuppressants and anti-cancer drugs); various proteins (e.g., short chain peptides, bone morphogenic proteins, glycoprotein and lipoprotein); cell attachment mediators; biologically active ligands; integrin binding sequence; ligands; various growth and/or differentiation agents (e.g., epidermal growth factor, IGF-I, IGF-II, TGF- β I-III, growth and differentiation factors, vascular endothelial growth factors, fibroblast growth factors, platelet derived growth factors, insulin derived growth factor and transforming growth factors, parathyroid hormone, parathyroid hormone related peptide, bFGF; TGF β superfamily factors; BMP-2; BMP-4; BMP-6; BMP-12; sonic hedgehog; GDF5; GDF6; GDF8; PDGF); small molecules that affect the upregulation of specific growth factors; tenascin-C; hyaluronic acid; chondroitin sulfate; fibronectin; decorin; thromboelastin; thrombin-derived peptides; heparin-binding domains; heparin; heparan sulfate; DNA fragments and DNA plasmids. If other such substances have therapeutic value in the orthopaedic field, it is anticipated that at least some of these substances will have use in the present invention, and such substances should be included in the meaning of "bioactive agent" and "bioactive agents" unless expressly limited otherwise.

"Biologically derived agents" include one or more of the following: bone (autograft, allograft, and xenograft) and derivatives of bone; cartilage (autograft, allograft, and xenograft), including, for example, meniscal tissue, and derivatives;

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ligament (autograft, allograft, and xenograft) and derivatives; derivatives of intestinal tissue (autograft, allograft, and xenograft), including for example submucosa; derivatives of stomach tissue (autograft, allograft, and xenograft), including for example submucosa; derivatives of bladder tissue (autograft, allograft, and xenograft), including for example submucosa; derivatives of alimentary tissue (autograft, allograft, and xenograft), including for example submucosa; derivatives of respiratory tissue (autograft, allograft, and xenograft), including for example submucosa; derivatives of genital tissue (autograft, allograft, and xenograft), including for example submucosa; derivatives of liver tissue (autograft, allograft, and xenograft), including for example liver basement membrane; derivatives of skin tissue; platelet rich plasma (PRP), platelet poor plasma, bone marrow aspirate, demineralized bone matrix, insulin derived growth factor, whole blood, fibrin and blood clot. Purified ECM and other collagen sources are also intended to be included within "biologically derived agents." If other such substances have therapeutic value in the orthopaedic field, it is anticipated that at least some of these substances will have use in the present invention, and such substances should be included in the meaning of "biologically derived agent" and "biologically derived agents" unless expressly limited otherwise.

"Biologically derived agents" also include bioremodelable collagenous tissue matrices. The expressions "bioremodelable collagenous tissue matrix" and "naturally occurring bioremodelable collagenous tissue matrix" include matrices derived from native tissue selected from the group consisting of skin, artery, vein, pericardium, heart valve, dura mater, ligament, bone, cartilage, bladder, liver, stomach, fascia and intestine, tendon, whatever the source. Although "naturally occurring bioremodelable collagenous tissue matrix" is intended to refer to matrix material that has been cleaned, processed, sterilized, and optionally crosslinked, it is not within the definition of a naturally occurring bioremodelable collagenous tissue matrix to purify the natural fibers and reform a matrix material from purified natural fibers. The term "bioremodelable collagenous tissue matrices" includes "extracellular matrices" within its definition.

"Cells" include one or more of the following: chondrocytes; fibrochondrocytes; osteocytes; osteoblasts; osteoclasts; synoviocytes; bone marrow cells; mesenchymal cells; stromal cells; stem cells; embryonic stem cells; precursor

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cells derived from adipose tissue; peripheral blood progenitor cells; stem cells isolated from adult tissue; genetically transformed cells; a combination of chondrocytes and other cells; a combination of osteocytes and other cells; a combination of synoviocytes and other cells; a combination of bone marrow cells and other cells; a combination of mesenchymal cells and other cells; a combination of stromal cells and other cells; a combination of stem cells and other cells; a combination of embryonic stem cells and other cells; a combination of precursor cells isolated from adult tissue and other cells; a combination of peripheral blood progenitor cells and other cells; a combination of stem cells isolated from adult tissue and other cells; and a combination of genetically transformed cells and other cells. If other cells are found to have therapeutic value in the orthopaedic field, it is anticipated that at least some of these cells will have use in the present invention, and such cells should be included within the meaning of "cell" and "cells" unless expressly limited otherwise. Illustratively, in one example of embodiments that are to be seeded with living cells such as chondrocytes, a sterilized implant may be subsequently seeded with living cells and packaged in an appropriate medium for the cell type used. For example, a cell culture medium comprising Dulbecco's Modified Eagles Medium (DMEM) can be used with standard additives such as non-essential amino acids, glucose, ascorbic acid, sodium pyrovate, fungicides, antibiotics, etc., in concentrations deemed appropriate for cell type, shipping conditions, etc.

"Biological lubricants" include: hyaluronic acid and its salts, such as sodium hyaluronate; glycosaminoglycans such as dermatan sulfate, heparan sulfate, chondroitin sulfate and keratan sulfate; synovial fluid and components of synovial fluid, including mucinous glycoproteins (e.g., lubricin), tribonectins, articular cartilage superficial zone proteins, surface-active phospholipids, lubricating glycoproteins I, II; vitronectin; and rooster comb hyaluronate. "Biological lubricant" is also intended to include commercial products such as ARTHREASETM high molecular weight sodium hyaluronate, available in Europe from DePuy International, Ltd. of Leeds, England, and manufactured by Bio-Technology General (Israel) Ltd., of Rehovot, Israel; SYNVISCO[®] Hylan G-F 20, manufactured by Biomatrix, Inc., of Ridgefield, New Jersey and distributed by Wyeth-Ayerst Pharmaceuticals of Philadelphia, Pennsylvania; HYLAGAN[®] sodium hyaluronate, available from Sanofi-Synthelabo, Inc., of New York, New York, manufactured by FIDIA S.p.A., of

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Padua, Italy; and HEALON® sodium hyaluronate, available from Pharmacia Corporation of Peapack, New Jersey in concentrations of 1%, 1.4% and 2.3% (for ophthalmologic uses). If other such substances have therapeutic value in the orthopaedic field, it is anticipated that at least some of these substances will have use
5 in the present invention, and such substances should be included in the meaning of "biological lubricant" and "biological lubricants" unless expressly limited otherwise.

"Biocompatible polymers" is intended to include both synthetic polymers and biopolymers (e.g., collagen). Examples of biocompatible polymers include: polyesters of [alpha]-hydroxycarboxylic acids, such as poly(L-lactide)
10 (PLLA) and polyglycolide (PGA); poly-p-dioxanone (PDS); polycaprolactone (PCL); polyvinyl alcohol (PVA); polyethylene oxide (PEO); polymers disclosed in United States Pat. Nos. 6,333,029 and 6,355,699; and any other bioresorbable and biocompatible polymer, co-polymer or mixture of polymers or co-polymers that are utilized in the construction of prosthetic implants. In addition, as new biocompatible,
15 bioresorbable materials are developed, it is expected that at least some of them will be useful materials from which the anchors may be made. It should be understood that the above materials are identified by way of example only, and the present invention is not limited to any particular material unless expressly called for in the claims.

"Biocompatible inorganic materials" include materials such as
20 hydroxyapatite, all calcium phosphates, alpha-tricalcium phosphate, beta-tricalcium phosphate, calcium carbonate, barium carbonate, calcium sulfate, barium sulfate, polymorphs of calcium phosphate, sintered and non-sintered ceramic particles, and combinations of such materials. If other such substances have therapeutic value in the orthopaedic field, it is anticipated that at least some of these substances will have use
25 in the present invention, and such substances should be included in the meaning of "biocompatible inorganic material" and "biocompatible inorganic materials" unless expressly limited otherwise.

It is expected that various combinations of bioactive agents, biologically derived agents, cells, biological lubricants, biocompatible inorganic
30 materials, biocompatible polymers can be used with the devices of the present invention.

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EXAMPLE 1

An aqueous suspension of SIS ("slurry") is made, of approximately 8 mg dry weight of comminuted SIS material per mL of water. The slurry is placed within a beaker on a stirring plate for approximately 3 minutes to ensure that the SIS is evenly dispersed in the suspension.

A polymer solution of 95:5 weight ratio solution of 60/40 PLA/PCL is made and poured into a flask. The flask is placed in a water bath, stirring at 60-70EC for 5 hrs. The solution is filtered using an extraction thimble, extra coarse porosity, type ASTM 170-220 (EC) and stored in flasks.

An equal amount of the polymer solution is added to the SIS slurry, to form an SIS/polymer mixture. The mixture is stirred on a stirring plate to make sure that the SIS and polymer are evenly dispersed in the suspension.

The porous scaffolds are obtained by freezing a comminuted SIS suspension at a slow, controlled rate ($-1^{\circ}\text{C}/\text{min}$ or less) to -20°C , followed by lyophilization, as follows: a slow-freeze ethanol bath is prepared by pouring enough ethanol to obtain about a 1 centimeter head in a flat-bottomed plastic container large enough to hold four 24-well culture plates. The container is placed in a -20°C freezer. Under a sterile hood using sterile conditions, an approximately 3ml aliquot of the comminuted SIS/polymer material is placed in each well of the tissue culture plates. The culture plates are then placed into the ethanol freeze bath and allowed to freeze overnight.

The frozen plates are then removed from the ethanol bath and placed in a suitable lyophilizer, such as the Virtis Genesis Series lyophilizer described above. The parameters used in the lyophilization process include a first period at a primary drying temperature of 13°C for 8 hours, followed by a second period at a secondary drying temperature of 35°C for 4 hours.

The resulting foam may be shaped or sculpted for the particular application. It is also understood that the mold could be provided in the desired shape, reducing or obviating the need for sculpting or trimming.

While Example 1 is directed to porous SIS/polymer scaffolds having a relatively large pore size, it is understood that the freezing and lyophilization profiles may be adjusted to produce scaffolds of desired size. Copending U.S. Application Serial No. XX/XXX,XXX entitled "Porous Extracellular Matrix Scaffold and

Method" (Attorney Docket No. 265280-71146, DEP-747), already incorporated by reference, provides various freezing and lyophilization profiles for the control of porosity.

5 **EXAMPLE 2**

 An SIS slurry and a polymer solution are prepared as in Example 1. However, rather than mixing a solution of polymer with the SIS, the solution of the polymer is layered over the mass. The layered mixture is then frozen and lyophilized as in Example 1, forming a foam having several layers of different mechanical and
10 biological composition. However, because the foam layers are formed together and expand somewhat into the interstices of the adjacent layer, there would not be a discrete demarcation between the synthetic foam and the ECM foam. Allowing the layers to mix slightly prior to lyophilization will increase the width of the transition zone between the layers.

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EXAMPLE 3

 In this example, a layered construct is formed wherein the scaffold has an SIS foam component and a synthetic mat component.

 An SIS slurry is prepared as in Example 1. Next, a 2 cm x 2 cm piece
20 of a 90/10 PGA/PLA mat is presoaked in water and then placed in the beaker with the SIS slurry. The 90/10 PGA/PLA piece is fully immersed within the slurry, resulting in an SIS-coated mat, having a thick coating of the SIS material on the synthetic mat. Several such SIS-coated 90/10 PGA/PLA mats are prepared. The coated mats are immediately transferred to a -80°C freezer. After freezing, the coated mats are
25 lyophilized as in Example 1.

 While 90/10 PGA/PLA is used for the mat in this example, it is understood that the additional structural component can be made of any biocompatible material, including bioabsorbable materials such as polylactic acid (PLA), polyglycolic acid (PGA), polycaprolactone (PCL), polydioxanone (PDO),
30 trimethylene carbonate (TMC), polyvinyl alcohol (PVA), copolymers or blends thereof. Furthermore, it is understood that additional structure may be provided by a variety of woven and nonwoven felts, meshes, textiles or other materials. As with the synthetic foam component, the composition of the additional structural component

may be selected to provide an appropriate resorption rate.

EXAMPLE 4

An SIS-coated 90/10 PGA/PLA mat is prepared as in Example 3. The
5 SIS-coated 90/10 PGA/PLA mat is "needled" using stainless steel needle pad (having
a plastic base with several closely spaced, about 1 mm apart, stainless steel needles).
The needling procedure involved applying the wet hybrid implant to the needle pad
and applying thumb pressure to drive the needles through the thickness of the implant.
The needling is believed to enhance mechanical entangling between the synthetic and
10 SIS portions of the hybrid scaffold, resulting in between adherence of the layers. The
implant is then transferred to the -80°C freezer, and subsequently lyophilized, as in
Example 3. Fig. 1 shows a similar foam made with a Vicryl™ mat. Fig. 2 is also
similar, having several layers of Vicryl™.

15 EXAMPLE 5

An SIS slurry is prepared as in Example 1. 2 cm diameter 90/10
PGA/PLA mats are placed into individual wells of a six well tissue culture plate.
4mL of the SIS slurry is pipetted onto each 90/10 PGA/PLA mat. The plate is
centrifuged at 2000 rpm for 2 minutes. The water is decanted off and another 2 mL of
20 the slurry is added and the plates are centrifuged in the same way. This treatment
resulted in an approximately 1 mm thick coating of SIS on the 90/10 PGA/PLA disk.
Some of these implants are needled in the same way as described in Example 4.
Others were not needled at all. The implants are then frozen and lyophilized as
described in Example 3. Fig. 3 shows a similar foam using Vicryl™.

25 As can be seen from the forgoing description, the concepts of the
present disclosure provide numerous advantages. For example, the concepts of the
present disclosure provide for the fabrication of a porous implantable scaffold which
may have varying mechanical properties to fit the needs of a given scaffold design.
For instance, the pore size and the material density may be varied to produce a
30 scaffold having a desired mechanical configuration. In particular, such variation of
the pore size and the material density of the scaffold is particularly useful when
designing a scaffold which provides for a desired amount of cellular migration
therethrough, while also providing a desired amount of structural rigidity.

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Additionally, by selecting an appropriate polymer, mechanical strength and resorption rates can be controlled, to provide mechanical support for a desired length of time subsequent to implantation.

While the disclosure is susceptible to various modifications and
5 alternative forms, specific exemplary embodiments thereof have been shown by way of example in the drawings and has herein be described in detail. It should be understood, however, that there is no intent to limit the disclosure to the particular forms disclosed, but on the contrary, the intention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the disclosure.

10 There are a plurality of advantages of the present disclosure arising from the various features of the apparatus and methods described herein. It will be noted that alternative embodiments of the apparatus and methods of the present disclosure may not include all of the features described yet still benefit from at least some of the advantages of such features. Those of ordinary skill in the art may
15 readily devise their own implementations of an apparatus and method that incorporate one or more of the features of the present disclosure and fall within the spirit and scope of the present disclosure.

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CLAIMS:

1. A method of making an implantable scaffold for repairing or regenerating body tissue, the method comprising the steps of:
 - 5 suspending ECM material in a liquid to form a slurry;
 - adding a synthetic portion to the slurry to make an ECM/synthetic composition;
 - freezing the composition to form crystals therein, and
 - driving off the crystals to form a foam.
- 10 2. The method of claim 1, wherein the ECM is comminuted.
3. The method of claim 2, wherein the ECM is selected from the group consisting of SIS, stomach submucosa, bladder submucosa, alimentary submucosa, respiratory submucosa, genital submucosa, and liver basement membrane.
- 15 4. The method of claim 2, wherein the synthetic portion is a synthetic mat, and further comprising the step of coating the mat with the comminuted ECM material, prior to the freezing step.
5. The method of claim 4, wherein the coating step includes
- 20 immersing the mat in the slurry.
6. The method of claim 4, wherein the coating step includes placing the slurry onto the mat and centrifuging the mat.
7. The method of claim 4, further comprising the step of needling the coated mat.
- 25 8. The method of claim 2, wherein the synthetic portion is a synthetic polymer solution.
9. The method of claim 8, wherein the synthetic polymer solution is mixed with the slurry to form an ECM/synthetic mixture.
10. The method of claim 9, further comprising the step of
- 30 coating a synthetic mat with the ECM/synthetic mixture.
11. The method of claim 8, wherein the synthetic polymer solution is layered on the slurry.

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12. The method of claim 11, wherein the foam has an ECM layer, a synthetic layer, and a transition zone having both ECM and synthetic fibers.
13. The method of claim 12, further comprising the step of allowing the ECM slurry and the polymer solution to mix slightly to increase the width of the transition zone.
14. The method of claim 2, further comprising the step of crosslinking the components of the foam.
15. The method of claim 2, wherein the driving off step is performed by lyophilization.
16. The method of claim 2, further comprising the step of adding an exogenous biologically active agent.
17. The method of claim 16, wherein the additional biologically active agent is added prior to the lyophilization step.
18. The method of claim 16, wherein the additional biologically active agent is added after the lyophilization step.
19. The method of claim 1, further comprising the step of shaping the foam.
20. A scaffold made according to the method of claim 1.
21. An implantable scaffold for repairing or regenerating body tissue, comprising:
a porous ECM foam, and
a synthetic mat embedded therein.
22. The scaffold of claim 21 wherein the porous ECM foam comprises comminuted SIS.
23. The scaffold of claim 22, wherein the synthetic mat is selected from the group consisting 90/10 PGA/PLA, 95/5 PLA/PGA, and PDO nonwoven mats, perforated thin sheets of 60/40 PLA/PCL, and perforated thin sheets of 65/35 PGA/PCL.
24. The scaffold of claim 21, wherein the porous foam further comprises an additional biologically active agent.
25. An implantable scaffold comprising
a mass of ECM intermixed with a fibrous synthetic portion in a composition dried to have a desired porosity.

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26. An implantable scaffold comprising
a porous foam comprising ECM and a synthetic portion distributed
within the foam.
27. The scaffold of claim 26, wherein the foam has a pore size of
5 100-600 microns.
28. The scaffold of claim 27, wherein the foam has a pore size of
300-600 microns.
29. The scaffold of claim 26, wherein the foam has a pore size of
5-90 microns.
- 10 30. The scaffold of claim 29, wherein the foam has a pore size of
about 20 microns.
31. The scaffold of claim 29, wherein the foam has a pore size of
about 80 microns.

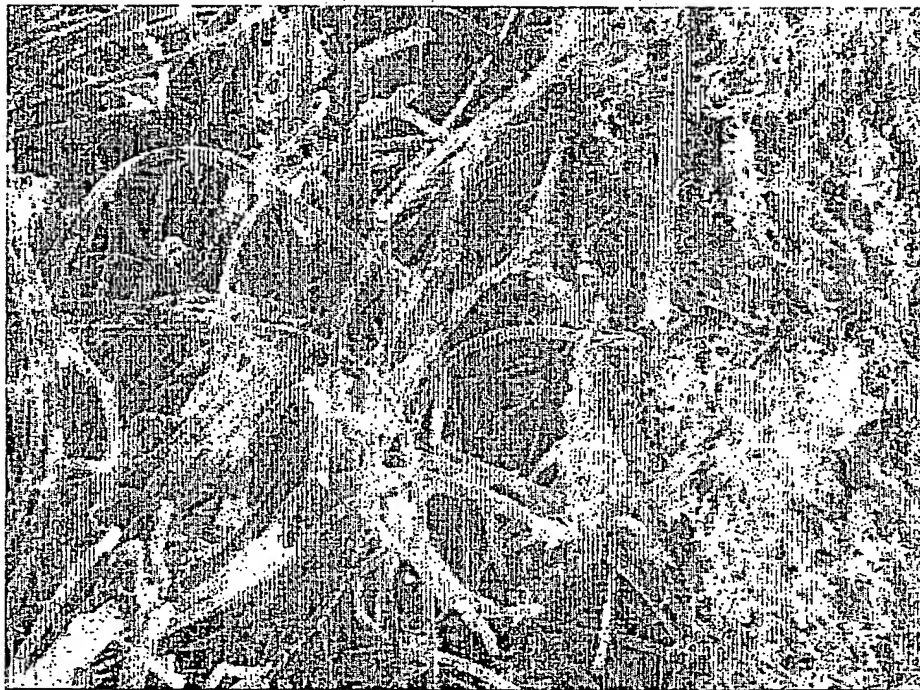


Fig. 1

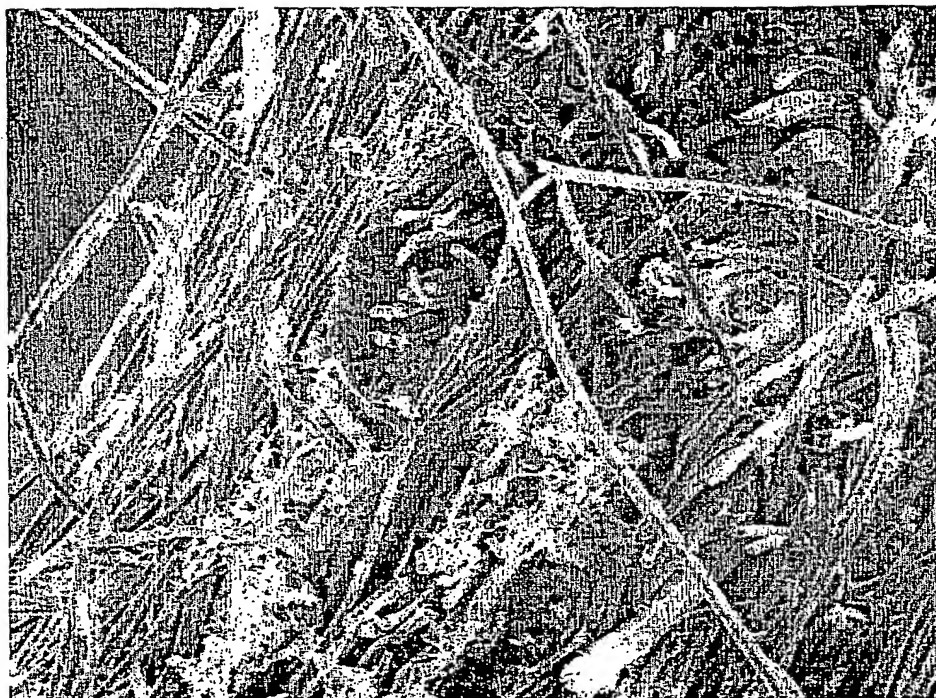


Fig. 2



Fig. 3

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